Effect of Carmustine (BCNU) on N-acetyltransferase Activity in Rat Glioma Cells

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Abstract

Rat glioma cell cytosol (subcellular fraction) was used to investigate the effect of carmustine (BCNU) on the activity of NAT toward p-aminiobenzoic acid and 2-amniofluorene using an acetyl coenzyme A (AcCoA) recycling assay and high pressure liquid chromatography (HPLC). BCNU is a cancer chemotherapeutic agent used in the treatment of brain tumor. Activity of N-acetyltransferase in rat glioma cell cytosol is found to be inhibited by BCNU. Its presence in the assay medium shows an inhibition in PABA-NAT activity about of 16.6% , 31.3% and 50% in rat glioma cells at 0.8 μM, 8 μM and 80 μM of BCNU concentrations respectively; while as AF-NAT activity decreased about 20.4%, 35.2% and 51.9% in rat glioma cells with 0.8 μM, 8 μM, and 80 μM of BCNU concentrations respectively.

The results of this study show that NAT activity is decreased by BCNU, which can be attributed to its anticancer activity.

Key words: Arylamine, N-acetyltransferase, Carmustine (BCNU), Glioma

Introduction

The arylamines represent one of the documented classes of chemicals known to induce tumor in humans[1,2]. N-acetylation is a major metabolic pathway of arylamine, and has been associated with carcinogen bioactivation. The reaction is catalyzed by cytosolic arylamine N-acetyltransferase (NAT), which required acetyl coenzyme A as a cofactor[3]. Acetyl coenzyme A-dependent arylamine NAT has been found in the tissues of several mammalian species[4-6]and in rat glioma cells[10]. Most of arylamine carcinogens, after undergoing metabolic activation, have been demonstrated to react with cellular DNA to form covalent DNA adducts, induce mutation and cause cancer in human[7-9]. Carmustine (BCNU) is commercially available nitrosourea derivative and chloroethyl nitrosourea compound which applied in antineoplastic therapy especially in central nervous system. Carmustine has been demonstrated to induce acute effect on glucose metabolism in human brain and glioblastoma and the hypermetabolic reaction possibly predicts longer survival in patients[12]. Carmustine has been reported to

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change the DNA in blood vessel endothelia in rat brain[13]. Carmustine also can increase survival in anaplastic gliomas patients[14]. There is no available information which addresses the effects of carmustine on NAT activity. Thus, the present study was performed to determine whether carmustine can affect NAT activity in rat glial tumor cells.

Materials and Methods

Chemicals and Reagents

Ethylenediaminetetraacetic acid (EDTA), 2-aminofluorene (AF), N-acetyl-2-aminofluorene (AAF), p-aminobenzoic acid (PABA), N-acetyl-p-aminobenzoic acid (N-Ac-PABA), acetylcarnitine, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), Tris, dimethyl sulfoxide (DMSO), dithiothreitol (DTT), Acetyl-Coenzyme A (AcCoA), and carnitine acetyltransferase were obtained from Sigma Chemical Co. (St. Louis, MO). All of the chemicals used were reagent grade.

Rat glioma cell line

Rat glioma cultured cell was obtained from the Veternary Hospital (Taipei, Taiwan). The cells were placed into 75 $cm^{[2]}$ tissue culture flasks and grown at 37 ℃ under a humidified 5% CO_2 atmosphere in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY), 2% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin).

Preparation of rat glioma cell cytosol

About 5 x 10^7 cells were placed in 2 ml of the lysis buffer (20 mM Tris/HCl, pH 7.5 (at 4 ℃), 1 mM DTT, 1 mM EDTA, 50 mM PMSF, and 10 mM leupeptin). The suspensions were centrifuged at 9000g for 1 min in a model 3200 Eppendorf/Brinkman centrifuge, and the supernatant fraction was subsequently centrifuged at 100,000g for 60 min. The supernatant was kept on ice for determinations.

Effect of carmustine on NAT activity in glioma cell cytosol

AcCoA-dependent N-acetylation of PABA and AF were determined as described by Hung et al[10]. The mixture in the assay system consisted of 90 μL cytosol, 50 μL buffer solution (20 mM Tris/HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 1 mM acetyl carnitine), AF or PABA as the substrates, and 10 μL of various concentration of BCNU (0.8 μM , 8 μM and 80 μM), adjusted to pH 7.5, and incubated at 37 ℃ for 10 min. The reaction was initiated by the addition of 20 μL AcCoA. The control was supplemented with 20 μL distilled water. The final concentration of PABA or AF was 0.1 mM, while that of AcCoA was 0.5 mM. The reaction mixture was incubated at 37 ℃ for 10 min and the reaction was stopped by the addition of 50 μL of 20% trichloroacetic acid for PABA reaction or 100 of acetonitrile for 2-AF reaction. All experiments were run in triplicates. The amounts of acetylated product and that of the remaining nonacetylated substrate were determined by high pressure liquid chromatography. Each sample of the NAT reaction mixture was injected into the C18 reversed-phase column (Spherisorb 4.6 x 250 nm) of a Beckman HPLC (pump 168 and detector 126), and eluted at a flow rate of 1.2 ml/min. For PABA and N-Ac-PABA, the solvent system was 50 mM acetic acid/CH_3CN (86:14) and detected was at 266 nm. The retention time was 4.0 min for PABA and 6.5 min for N-Ac-PABA. For AF and AAF, the solvent system was 20 mM KH_2PO_4(pH 4.5)/CH_3CN (53:47) with detection at 280 nm. The retention time was 6.5 min for AAF and 9 min for AF. All compounds were quantified by comparing the integrated area of the elution peak with that of the known amount of standards. NAT activity is expressed as nmol acetylated substrate per min per mg of cytosolic protein. The protein concentration of the tissue cytosol was determined by the method of Bradford[11], with bovine serum albumin as the standard. All samples were assayed in triplicates.

Results

The PABA-NAT activity and AF-NAT activity in glioma cell cytosol are decreased by BCNU in this study. Activity of N-acetyltransferase in rat glioma cell cytosol is found to be inhibited by BCNU. Its presence in the assay medium shows an inhibition in PABA-NAT activity about of 16.6%, 31.3% and 50% in rat glioma cells at 0.8 μM, 8 μM and 80 μM of BCNU concentrations respectively; while as AF-NAT activity decreased about 20.4%, 35.2% and 51.9 % in rat glioma cells with 0.8 μM, 8 μM and 80 μM of BCNU concentrations respectively (Table 1).

Discussion

The initial choice of 2-aminofluorene (AF) and p-aminobenzoic acid (PABA) as test substrates was based on previous studies in mice [15] and current interest in comparing the metabolism of a carcinogen (AF) to a non-carcinogen (PABA). In mammals there are at least two arylamine N-acetyltransferase, NAT1 and NAT2, which show differences in substrate specificities. In mice, NAT1 does not catalyze the acetylation of PABA, but has activity for AF. Mouse NAT2 catalyzes acetylation of both PABA and AF[16]. In contrast, human NAT1 acetylates PABA and AF, but human NAT2 acetylates AF but not PABA[17]. NAT in human can be divided into slow and rapid acetylator phenotypes[3]. Variations in the activity, and affinity of the rapid and slow acetylator isoenzymes for aromatic amines substrates appear to account for the difference between the rapid and slow pathways of the in vivo acetylation. Interest in polymorphic acetylation of carcinogenic arylamines in human is largely due to the statistical linking of slow acetylator phenotypes with known occupational exposure to compounds which caused higher risk for bladder cancer[4,18], and rapid acetylator phenotypes to a higher risk for colorectal cancer[19,20]. N-acetyltransferase plays a key roles on the genotoxic effetcs of the heterocyclic aromatic amines. Metabolites of arylamine have been demonstrated to react with cellular DNA to form covalent DNA adducts, induce mutation and cause cancer in human[7-9]. Carcinogen-DNA adduct formation is an important step in chemical carcinogenesis[21,22]. BCNU is a potent chemotherapeutic drugs and have been used extensively for treatment of brain tumor[23,24]. The present study indicates that the NAT activity is presented in rat glioma cell cytosol and BCNU could decrease NAT activity in rat glioma cytosol. NAT activity in normal rat glial cell cytosol should be studied in further when normal rat glial cell could be isolated to culture in the future.

In summary, the present studies show that NAT activity was inhibited by BCNU in rat glial tumor cells, which can be attributed to its anticancer activity.

Table 1. Effects of carmustine on NAT activity in cytosols of rat glioma

Concentration of Carmustine	In cytosols	
	AAF (nmol/min/mg)(#%)	N-Ac-PABA (nmol/min/mg)(#%)
Control	1.08 ± 0.18(100%)	0.96 ± 0.16(100%)
0.8 μM	0.86 ± 0.16(79.6%)	0.80 ± 0.14(83.4%)
8 μM	[a]0.70 ± 0.13(64.8%)	[a]0.66 ± 0.10(68.7%)
80 μM	[b]0.52 ± 0.10(48.1%)	[b]0.48 ± 0.09(50%)

Values are mean ± S.D. n=12

[a]differs between 8 μM carmustine and control. $p<0.05$

[b]differs between 80 μM carmustine and control. $p<0.01$

#% of control

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Carmustine 對大白鼠大腦膠質瘤細胞株中乙醯轉移酵素活性的影響

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摘 要

芳香胺化合物經 N 基乙醯轉移酵素 (N-acetyltransferase) 之 N 基乙醯化可誘發癌，本研究是 in vitro 探討 carmustine(BCNU) 對大白鼠大腦膠質瘤細胞株中乙醯轉移酵素活性 (NAT) 的影響。取 12 個大白鼠大腦膠質細胞瘤組織用乙醯化輔酶 A 作乙醯基而再利用高壓液相層析儀分析乙醯轉移酵素的活性及 BCNU 對大白鼠大腦膠質細胞瘤組織中乙醯轉移酵素活性的影響，以 2-aminofluorene(2-AF) 和 p-aminobenzoic acid(PABA) 作受質。

結果顯示白鼠大腦膠質瘤細胞株中對 AF 及 PABA 受質皆有乙醯轉移酵素的活性存在，其對 AF 及 PABA 的活性平均值各爲 1.08 ± 0.18 及 0.96 ± 0.16 nmol/min/mg protein。在 0.8 μM, 8 μM and 80 μM 的 carmustine 濃度中，白鼠大腦膠質瘤細胞株中 PABA-NAT 活性降低約爲 16.6%, 31.3% and 50% ，AF-NAT 活性降低約爲 20.4%, 35.2% and 51.9%。

Carmustine 降低白鼠大腦膠質瘤細胞株中乙醯轉移酵素的活性的作用，可能是 carmustine 治療膠質細胞瘤的一種機轉。本實驗報告是 in vitro 探討的初步報告，當然須再進一步 in vivo 的研究確認。

關鍵詞： 芳香胺化合物，N 基乙醯轉移酵素，膠質細胞瘤，carmustine (BCNU)

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